Characterization of Protein Fractions from Immature Alaska Walleye Pollock (*Theragra chalcogramma*) Roe

P.J. Bechtel, J. Chantarachoti, A.C.M. Oliveira, and S. Sathivel

ABSTRACT: There are good markets for mature pollock roe; however, immature pollock roe is underutilized. The physical and nutritional properties of immature pollock roe (IPR) have not been reported, which limits its potential use as a food ingredient. The objective of this study was to evaluate the physical and nutritional properties of immature pollock roe and soluble and insoluble protein powders made from the immature roe. IPR samples were obtained during the spring pollock harvest from a seafood processing plant in Kodiak, Alaska. Soluble (SP) and insoluble protein (IP) fractions were produced by heating IPR, separating by centrifugation and freeze drying. The protein contents of freeze-dried IPR, SP, and IP were 81.7%, 63.2%, and 83.0%, respectively. The amino acid contents of IPR and IP were similar except for isoleucine and valine. However, the amino acid contents of IPR and IP were different from values for SP. Lipid contents of IPR, SP, and IP were 9.2%, 9.3%, and 11.1%, respectively. Palmitic acid (C16:0; 21.2%), DHA (C22:6ω3; 21.2%), and EPA (C20:5ω3; 19.0%) were the 3 most abundant fatty acids in fresh IPR. Fat adsorption capacity value for SP was significantly higher than IPR and IP ($P < 0.05$). SDS electrophoresis indicated a major protein band with molecular weight of 103 KDa in all samples. Results indicate that IPR can be utilized to make a number of unique food ingredients with good nutritional characteristics and functional properties.

Keywords: fish protein, immature pollock roe, protein powder, roe characterization

Introduction

Forty percent of the total seafood harvested in the United States comes from the North Pacific groundfish fishery (SCS 2004). The walleye pollock (*Theragra chalcogramma)* fishery alone constitutes about 71.8% of the average groundfish harvested in Alaska (AFSC 2005). Pollock roe is an important export product, accounting for 27577 MT of the total fishery products exported from United States in 2005 (NMFS 2005). Alaska walleye pollock is used in the manufacturing of surimi, frozen fish sticks, breaded fillets for fast food fish sandwiches, and imitation crab (Pemberton 2003). Pollock roe is processed into Tarako (salted roe) or Mentaiko (roe seasoned with salt, sugar, and monosodium glutamate) and can be used as an ingredient in salad dressing, soups, and sauces (Bledsoe and others 2003). The grade of Alaska walleye pollock roe is determined by color, size, maturity, and firmness (Chou 2004). There are significant quantities of immature pollock roe (IPR) harvested annually that are discarded or processed into fish meal. Immature pollock roe is a potential raw material for the manufacture of protein powders with distinct nutritional characteristics.

Protein powders from fish by-products are good sources of high-quality protein with many desirable functional properties. Protein powders from fish by-products could potentially be used in foods as binders and emulsifiers (Sathivel and others 2004). In addition, fish protein is regarded as an excellent source of high-quality protein, with high concentration of the essential amino acids lysine and methionine.

The physical and nutritional properties of IPR have not been reported, which limits its potential use as a food ingredient. The objective of this study was to evaluate the physical and nutritional properties of IPR and the soluble and insoluble protein powders made from this material.

Materials and Methods

Alaska walleye pollock roe sampling

Alaska walleye pollock roe samples were obtained in March 2004 from a seafood processing plant in Kodiak, Alaska. The fresh roe was collected from the by-product line and transported to the Fishery Industrial Technology Center. Samples were placed in vacuum bags and frozen immediately at –20 °C until processing. Samples were trimmed, ground, and separated into 3 replicates. Processed samples were stored at –70 °C for analysis.

Roe protein powder preparations

The protein extraction procedure adapted from Sathivel and others (2004) was repeated 3 times. Samples were store frozen for 2 mo and then a 300-g portion of each ground IPR sample was mixed with 300-mL distilled water and homogenized in a blender model 51BL31 (Waring Products, Torrington, Conn., U.S.A.) for 2 min. The mixture was heated at 80 to 85 °C and held at this temperature for 60 min with stirring. Then 300 mL of distilled water was added and homogenized for 15 s. The heated suspension was centrifuged with centrifuge model J2-HS (Beckman Coulter Inc., Fullerton, Calif., U.S.A.) at 5660 × *g* for 15 min at 23 °C, resulting in 3 separate phases. The fat layer (top) was discarded. The 2nd layer (soluble protein; SP), the bottom pellet (insoluble protein; IP), and a 200 g portion of the initial roe sample (total protein; IPR) were freeze dried. The resulting

MS20070191 Submitted 3/13/2007, Accepted 4/18/2007. *Author Bechtel is with Dept. of USDA-ARS-Pacific West Area, Subarctic Agricultural Research Unit, Univ. of Alaska Fairbanks, 245 O'Neill Bldg., Fairbanks, AK 99775-7220, U.S.A. Authors Chantarachoti, Oliveira, and Sathivel are with the Fishery Industrial Technology Center, Univ. of Alaska Fairbanks, 118 Trident Way, Kodiak, AK 99615, U.S.A. Direct inquiries to author Bechtel (E-mail: bechtel@sfos.uaf.edu).*

doi: 10.1111/j.1750-3841.2007.00396.x

roe protein powders were kept in plastic bottles and stored at –80 °C until analyzed.

Proximate composition and lipid extraction

Moisture and ash content were determined according to AOAC methods 952.08 and 938.08, respectively (Helrich 1990). Protein analyses were carried out using a protein analyzer LECO model FP2000 (LECO, St. Joseph, Mich., U.S.A.). Lipid content was determined by the method of Folch and others (1957). Lipids were stored under a headspace of nitrogen in amber screw cap vials at –80 °C until analyzed for fatty acids, lipid classes, and cholesterol content. All analyses were performed in triplicates.

Fatty acid profile and gas chromatography (GC) analysis

Fatty acids were prepared according to the procedure of Maxwell and Marmer (1983) using C23:0 as internal standard. Fatty acid methyl esters were analyzed on a GC model 6850 (Agilent Technologies, Wilmington, Del., U.S.A.) fitted with a DB-23 (60 m × 0.25 mm id., 0.25 μm film) capillary column (Agilent Technologies). Data were collected and analyzed using the GC ChemStation program (Rev.A.08.03 [847]; Agilent Technologies 1990–2000). Hydrogen was used as carrier gas at constant flow of 1.0 mL/min with an average velocity of 30 mL/min. The initial nominal pressure of the inlet was 15.3 psi and both injector and detector were held at a constant temperature of 275 °C. The split ratio was 25:1 and the oven programming was 140 to 200 °C at a rate of 2 °C/min, 200 to 220 °C at a rate of 1 °C/min, and 220 to 240 °C at a rate of 10 °C/min for a total run time of 52 min. The detector was operated at a constant makeup flow of 35 mL/min of nitrogen, with an air and hydrogen flow of 450 mL/min and 40 mL/min, respectively. An autosampler performed the GC injections of standards and sample, and injection volume was 1 μL. The ChemStation enhanced integrator program was used to integrate the chromatogram peaks. All standards used in the identification of peaks were purchased from Supelco (Bellefonte, Pa., U.S.A.). The standards used were Supelco 37, Bacterial Acid Methyl Esters Mix, Marine Oil nr 1, and Marine Oil nr 3. Samples were run in triplicates, and cod liver oil was used as a secondary reference standard (Ackman and Burgher 1965).

Lipid classes analysis

A Iatroscan TLC/FID analyzer model MK-6s (Iatron Laboratories Inc., Tokyo, Japan) was used to determine the distribution of the main lipid classes in the extracted lipids as described by Oliveira and Bechtel (2005). Seven standards obtained from Sigma (St. Louis, Mo., U.S.A.) were used to identify the lipid classes and included cholesterol (ST), tripalmitin (TAG), palmitic acid (FFA), L-α- phosphatidylcholine (PL), 1,2-dipalmitoyl-*sn*-glycerol (1,2-DAG), 1,3 dipalmitoyl-*sn*-glycerol (1,3-DAG), and DL-α- monopalmitoylglycerol (MAG). The solvent system used was a mixture of hexane: ethyl ether:formic acid at the ratio of 80:25:1.2. In this system 1,3-DAG coelutes with the sterol peak and is separated from the 1,2-DAG peak. Lipid classes, analyzed in triplicates, were reported as percent triacylglycerides, 1,2-diacylglycerides, monoacylglycerides, free fatty acids, phospholipids, and the combined percentages of sterols and 1,3-diacylglicerides.

Cholesterol analysis

Duplicate analyses were performed on the lipid extracts from immature pollock roe samples. The analysis followed the method of Kovacs and others (1979) with modifications as described by Oliveira and Bechtel (2006). Chromatography analysis was carried out on a GC 6850N (Agilent Technologies) coupled to an FID and fitted with a DB-17 (30 m × 0.25 mm × 0.15 μm film) capillary column (Agilent Technologies). Chromatographic conditions were as described by Oliveira and Bechtel (2006). A 5-point calibration curve was determined ($R^2 = 0.99$) using the peak area ratio of cholesterol (0.1 to 1 mg) and 5-α-cholestane (1 mg) compared with the weight ratio of these compounds.

Amino acid and mineral analysis

The amino acid analysis was conducted in triplicates by the AAA Service Laboratory Inc. (Boring, Oreg., U.S.A.). All samples were hydrolyzed with 6N HCl and 2% phenol at 110 °C for 22 h. Amino acids were quantified using a Beckman 6300 analyzer with a postcolumn ninhydrin derivatization. Tryptophan and cysteine contents were not determined. Samples for mineral analysis were ashed overnight at 550 °C. The ash residues were digested overnight in an aqueous solution containing 10% (v/v) hydrochloric acid and 10% (v/v) nitric acid. Digested solutions were diluted and analyzed for P, K, Ca, Mg, Na, Cu, Zn, Sr, Fe, Mn, Cd, Ni, Pb, Ag, Hg, and As using an inductively coupled plasma optical emission spectroscopy (Perkin Elmer Optima 3000 Radial ICP—OES; PerkinElmer Life And Analytical Sciences Inc., Boston, Mass., U.S.A.).

Physical and functional properties

Color flex model 45/0 (Hunter Associates Laboratory Inc., Reston, Va., U.S.A.) was used to measure the color values in CIE $L^*a^*b^*$ color scale. L^* describes the lightness of the sample, a^* intensity in red ($a^* > 0$), and b^* intensity in yellow ($b^* > 0$). Values were determined in triplicates for every sample. Percent protein solubility at 30 °C was determined in triplicate for every sample by a modification (Bechtel 2003) of the protein dispersibility index (Batal and others 2000).

Emulsifying stability (ES) was evaluated in triplicate according to the method of Yatsumatsu and others (1972). A 500-mg sample was transferred into a 250-mL beaker and dissolved in 50 mL of 0.1M NaCl, and then 50 mL of soybean oil was added. The homogenizer, equipped with a motorized stirrer controlled by a rheostat, was immersed to half the depth of the mixture, and operated for 2 min at 100% output at 120 V to make an emulsion. From the emulsion, three 25-mL aliquots were immediately taken and transferred into three 25-mL graduated cylinders. The emulsions were allowed to stand for 15 min at 25 °C and then the aqueous volume was read. ES (percent) was calculated as [(total volume – aqueous volume)/ total volume] × 100.

The fat adsorption capacity (FA) of the samples was determined in triplicate by placing 500 mg of sample into a 50-mL centrifugal tube and adding 10 mL of soybean oil (Shahidi and others 1995). The sample was thoroughly mixed with a small steel spatula, kept for 30 min at 25 °C with intermittent mixing every 10 min, and then centrifuged at 2560 × *g* for 25 min. The free oil was then decanted and the fat absorption of the sample was determined from the weight difference. FA was expressed in terms of milliliters of fat adsorbed by 1 g of protein.

Sodium dodecyl sulfate tricine/polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE system of Schagger and von Jagow (1987) was used with a Photodyne Foto/Force 300 apparatus under reducing conditions. Novex 10% to 20% precast tricine gels (Invertogen Inc., Carlsbad, Calif., U.S.A.) were used. The molecular mass standards were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.) and protein bands were visualized with Coomassie blue staining. This analysis was not replicated.

Statistical analysis

The means and standard errors are derived from an analysis of variance run on Statistica version 6.0 (StatSoft Inc., Tulsa, Okla., U.S.A.). All data sets were tested for normality. For the normally distributed data, Duncan's posthoc test was use to determined statistical significance ($P < 0.05$). In case of protein and lipid contents, certain minerals (K, Ca, Mg, Na, Zn), and color (L^*, a^*, b^*), normality assumptions were not met and, hence, the data were subjected to a nonparametric test (Kruskal–Wallis test).

Results and Discussion

Proximate analysis

Table 1 lists the protein content of fresh immature walleye pollock. Fresh immature pollock roe had a low lipid level of 0.6% and a high protein content of 27.5%. There are no values in the literature for the proximate analysis of immature pollock roe; however, Iwasaki and Harada (1985) published the proximate analysis of roe from 14 common marine species and reported that the protein content of roe from chum salmon, Pacific flounder, and Pacific cod had similar percent protein values ranging from 26.5% to 27.2%. The fat content of the IPR was 0.6%, which is much lower than the range of values reported by Iwasaki and Harada (1985) for the lipid content of roe from marine fin fish (3.0% to 19.8%).

Fresh IPR was homogenized in water, heated to 85 °C, and centrifuged to create soluble (SP) and insoluble (IP) protein fractions (Sathivel and others 2004), which were subsequently freeze-dried. The proximate analyses of freeze-dried IPR (FD-IPR), SP, and IP are listed in Table 1. The major constituents of FD-IPR were 81.7% protein and 9.2% lipid. The protein compositions of the soluble protein (SP) and insoluble protein (IP) fractions were 63.2% and 83.0%, respectively, and the lipid contents of the 2 fractions were 9.3% and 11.1%. The compositions of FD-IPR and IP were similar; however, the SP fraction had a large ash content, which was attributed to large contents of potassium, phosphorus, and sodium that were concentrated during fractionation and the subsequent freeze-drying process.

Fatty acid profile

Hexadecanoic acid (palmitic; C16:0; 21.2%), docosahexaenoic acid (DHA; C22:6ω3; 21.2%), eicosapentaenoic acid (EPA; C20:5ω3; 19.0%), and (oleic; C18:1ω9 cis; 11.6%) were the 4 most abundant fatty acids found in IPR. Oliveira and Bechtel (2005) evaluated the fatty acid composition in lipids extracted from pollock by-products, including heads, skin, frames, and viscera, and found the same 4 fatty acids to be the most abundant in the extracted oils of these tissues. The authors determined that pollock frames, heads, and skin all had fat contents below 1.2% and that the content of EPA in these tissues ranged from 12.3% to 16.9% and DHA from 11.8% to 18.5% (Oliveira and Bechtel 2005). In this study the fat content of fresh IPR was below 0.6% with the combined EPA and DHA contents being higher (40.2%) than the ranges determined by Oliveira and Bechtel (2005) for other walleye pollock byproducts.

Percent values of saturated fatty acids (SAT), monounsaturated fatty acids (MUSA), polyunsaturated fatty acids (PUFA), SAT to PUFA ratio, omega 3 (ω-3), omega 6 (ω-6), and the ratio of ω-3/ω-6 found in IPR were 26.1, 28.8, 44.5, 0.6, 42.9, 1.4, and 29.8, respectively (Table 2). Tocher and Sargent (1984) also reported high values for total ω-3 fatty acids (38.2% to 50.3%) and low ω-6 values (1.3% to 3.5%) for ripe herring, cod, haddock, whiting, saithe, sand eel, and capelin roes, which overlap the values found in this study for these classes of fatty acids. Additionally, Tocher and Sargent (1984) analyzed the fatty acid composition of the polar (phospholipids) and neutral (mainly triacyglycerides) lipid fractions of roe from these fish species, and reported that the phospholipids fraction had high levels of DHA (21.2% to 29.4%) and EPA (11.4% to 18.3%). Kaitaranta and Linko (1984) examined the fatty acid composition during maturation of Baltic herring, roach, perch, burbot, and rainbow trout roes and reported that the fatty acid compositions change little during maturation.

Lipid classes and cholesterol content

The phospholipids class was the most abundant in IPR (71.5%), which is consistent with the high values of EPA and DHA reported in Table 2. Oliveira and Bechtel (2005) reported percent phospholipids in pollock heads, skin, and frames to range from 34.5% to 61.8%,

Table 1—Proximate analysis (% w/w) of immature pollock roe (IPR) and the soluble and insoluble protein fractions of IPR

	Protein	Ash	Moisture	Lipid
Fresh IPR	27.49 ± 0.03	1.40 ± 0.01	68.28 ± 0.04	0.57 ± 0.01
FD IPR	81.74 ± 0.45[a,b]	4.70 ± 0.06[b]	2.96 ± 0.03[b]	9.15 ± 0.19[a]
SP	63.16 ± 2.34[a]	18.39 ± 0.19[c]	8.38 ± 0.07[c]	9.29 ± 0.16[a]
IP	82.97 ± 0.22[b]	2.38 ± 0.12[a]	2.20 ± 0.08[a]	11.14 ± 0.52[a]

Mean values and SE presented in the table.
[a,b,c]Different letters within a column indicate statistical difference at $P < 0.05$; IPR, immature pollock roe; FD, freeze dried; SP, soluble protein fraction of IPR; IP, insoluble protein fraction of IPR.

Table 2—Fatty acid analysis of immature pollock roe (percent of total oil extracted)

Fatty acid	Fresh IPR
C14:0	2.46 ± 0.04
C15:0	0.26 ± 0.00
C16:0	21.23 ± 0.05
C16:1ω9	0.35 ± 0.01
C16:1ω7	4.78 ± 0.06
C16:1ω5	0.28 ± 0.01
C17:1ω9	1.22 ± 0.05
C18:0	2.14 ± 0.01
C18:1ω11	0.54 ± 0.01
C18:1ω9 *cis*	11.56 ± 0.13
C18:1ω7	5.93 ± 0.02
C18:1ω5	0.21 ± 0.00
C18:2ω6 *cis*	0.57 ± 0.02
C18:3ω4	0.21 ± 0.00
C18:3ω3	0.21 ± 0.00
C18:4ω3	0.69 ± 0.01
C20:1ω11	1.32 ± 0.03
C20:1ω9	1.07 ± 0.00
C20:1ω7	0.21 ± 0.02
C20:4ω6	0.87 ± 0.00
C20:4ω3	0.34 ± 0.01
C20:5ω3	18.98 ± 0.12
C22:1ω11	0.81 ± 0.03
C22:1ω7	0.47 ± 0.01
C22:5ω3	1.47 ± 0.01
C22:6ω3	21.17 ± 0.28
FAME ID	99.38 ± 0.03
SAT (S)	26.10 ± 0.03
MUSA	28.76 ± 0.33
PUFA (P)	44.52 ± 0.34
S/P	0.59 ± 0.01
ω-3	42.87 ± 0.36
ω- 6	1.44 ± 0.02
ω-3/ ω-6	29.75 ± 0.68

Mean values and SE presented in the table.
FAME ID fatty acid methyl esters identified; SAT saturated fatty acids; MUSA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; S/P ratio of saturated to polyunsaturated fatty acids.

which are lower than found for IPR in this study. There is large variation in the percentage of polar lipids found in roe from different fish species (Kaitaranta and Ackman 1981; Tocher and Sargent 1984; Body 1989; see review by Bledsoe and others 2003). Values range from 86.4% for Baltic herring roe to a low of 12.6% for burbot (Kaitaranta and Ackman 1981). High values for polar lipid classes (61.2% to 86.4%) of mature roe have been reported for marine species, including cod, Baltic herring, haddock, whiting, and saithe (Kaitaranta and Ackman 1981; Tocher and Sargent 1984). Kaitaranta and Ackman (1981) provided evidence that percent phospholipids increased in Baltic herring and rainbow trout as the roe matured.

The monoacylglycerides and 1,2-diacylglycerides were close to negligible in IPR, while the 2nd most abundant lipid class was the combined sterols and 1,3-diacylglycerides component at 15.0%. The procedure used in this study to separate lipid classes did not resolve sterols from 1,3-diacylglycerides, thus the need to determine cholesterol content in IPR. The cholesterol content in IPR was determined in duplicate with the average value of 17.5 g/100 g oil. This value may seem rather high when compared to values reported by Bledsoe and others (2003). The authors reported cholesterol values as a percentage of total lipids for a large number of mostly mature roe products, and these ranged from a low of 0.5% to 0.8% for roach and burbot to a high of 11% to 13.5% for whiting, saithe, and orange roughy. However, when the cholesterol content of IPR is expressed per 100 g of tissue the value is approximately 100 mg of cholesterol/100 g IPR. We attribute this difference to the imprecision of the lipid class' analysis when estimating the distribution of all 6 lipid classes listed in the Materials and Methods section.

Amino acid and mineral composition

The amino acid contents of IPR and IP were similar except for isoleucine (ILE) and valine (VAL), which were different ($P < 0.05$), as shown in Table 3. The TEAA content of both IPR and IP was similar and both were high at 43.4 and 43.9, respectively. The TEAA value for SP (33.2) was much lower than for IPR and IP, reflecting lower protein quality. The SP fraction contains higher levels ($P < 0.05$), of GLU, GLY, HIS, PRO, SER, and TYR, which contribute to the higher TNEAA value. Levels of MET for IPR, IP, and SP ranged from 2.53 to 2.88, and from 8.23 to 8.87 for LYS. One criterion of protein quality is the ratio of TEAA/TNEAA, and these values were much higher for IP (0.78) and IPR (0.77) than for SP (0.51).

The amino acid composition for roe from 21 aquatic animal species including Alaska pollock was summarized by Bledsoe and others (2003). The ratio of TEAA/TNEAA for roe from the marine fin fish ranged from a low value of 0.67 for mullet to a high of 0.82 for chum salmon. The value obtained in this study for immature pollock roe of 0.77 was close to the value of 0.74 reported for mature pollock roe by Bledsoe and others (2003). In addition, the amino acid compositions reported by this authors were similar as determined in this study. There are no studies on the amino acid composition of pollock roe as it matures; but Kaitaranta and others (1980) reported that the amino acid composition of both Baltic herring roe and rainbow trout roe did not change during maturity. Iwasaki and Harada (1985) compared the proximate analysis and amino acid composition of roe and muscle from 14 Pacific marine species of fin fish. The TEAA/TNEAA ratios of the roe were greater than TEAA/TNEAA ratios for comparable muscle in 8 of the 14 species.

The calcium content was low in all samples (0.02% to 0.09% dry weigh) but the levels of P ranged from 0.9% to 3.2% (Table 4). Although not always significantly different ($P < 0.05$), levels of most minerals, including P, K, Mg, Na, and Cu, were highest in the SP fraction and lowest in the IP fraction. This indicates that these minerals were associated with the aqueous phase during the separation procedures. Zinc, on the other hand, was the only mineral to have higher levels ($P < 0.05$) in the IP when compared to the SP fraction. Levels of Cd, Pb, Hg, and As were very low in the IPR. There are little data available on the mineral content of pollock roe; however, Rodrigo and others (1998) have reported the mineral composition of salted dried roes of hake and ling.

Table 3—Amino acid composition (AA wt/total AA wt %) of immature pollock roe (IPR) and the soluble and insoluble protein fractions of IPR

	IPR	SP	IP
Alanine (ALA)	7.03 ± 0.04[b]	5.93 ± 0.02[a]	7.16 ± 0.06[b]
Arginine (ARG)	5.71 ± 0.02[a]	5.50 ± 0.11[a]	5.73 ± 0.05[a]
Aspartic acid (ASP)	8.52 ± 0.05[b]	6.53 ± 0.04[a]	8.70 ± 0.10[b]
Glutamic acid (GLU)	12.75 ± 0.17[a]	17.41 ± 0.21[b]	12.42 ± 0.22[a]
Glycine (GLY)	2.97 ± 0.02[a]	4.08 ± 0.01[b]	2.96 ± 0.02[a]
Histidine (HIS)	2.42 ± 0.03[a]	2.57 ± 0.04[b]	2.34 ± 0.02[a]
Isoleucine (ILE)	5.82 ± 0.03[b]	2.97 ± 0.01[a]	5.98 ± 0.05[c]
Leucine (LEU)	9.72 ± 0.05[b]	6.31 ± 0.02[a]	9.90 ± 0.07[b]
Lysine (LYS)	8.23 ± 0.04[a]	8.87 ± 0.11[b]	8.23 ± 0.07[a]
Methionine (MET)	2.86 ± 0.01[b]	2.53 ± 0.04[a]	2.88 ± 0.02[b]
Phenylalanine (PHE)	5.25 ± 0.24[a]	4.15 ± 0.52[a]	5.13 ± 0.30[a]
Proline (PRO)	5.97 ± 0.02[a]	7.03 ± 0.16[b]	5.81 ± 0.08[a]
Serine (SER)	5.70 ± 0.03[a]	9.91 ± 0.11[b]	5.48 ± 0.07[a]
Threonine (THR)	4.99 ± 0.06[b]	4.38 ± 0.05[a]	5.11 ± 0.07[b]
Tyrosine (TYR)	5.47 ± 0.04[a]	6.18 ± 0.19[b]	5.47 ± 0.07[a]
Valine (VAL)	6.52 ± 0.06[b]	4.01 ± 0.01[a]	6.71 ± 0.04[c]
TEAA	43.39	33.23	43.94
TNEAA	56.54	65.14	56.07
TAA	99.93	100.01	98.37

Mean values and SE presented in the table.
[a,b,c]Different letters within a row indicate statistical difference at $P < 0.05$; IPR immature pollock roe; SP soluble protein fraction of IPR; IP insoluble protein fraction of IPR.
TEAA is total essential amino acids (ILE, LEU, LYS, MET, PHE, THR, VAL); TNEAA is total nonessential amino acids; TAA is total amino acids.
Tryptophan and cysteine were not determined.

Table 4—Mineral composition of immature pollock roe (IPR) and the soluble and insoluble protein fractions of IPR

Percent dry weight	IPR	SP	IP
Phosphorus (P)	1.10 ± 0.01[b]	3.19 ± 0.04[c]	0.85 ± 0.02[a]
Potassium (K)	0.66 ± 0.01[a,b]	3.98 ± 0.08[b]	0.21 ± 0.01[a]
Calcium (Ca)	0.03 ± 0.00[a,b]	0.09 ± 0.00[b]	0.02 ± 0.00[a]
Magnesium (Mg)	0.04 ± 0.00[a,b]	0.14 ± 0.00[b]	0.02 ± 0.00[a]
Milligram/kilogram dry weight			
Sodium (Na)	4212.7 ± 70.34[a,b]	22586.7 ± 2740.65[b]	1419.7 ± 90.35[a]
Copper (Cu)	3.19 ± 0.15[a]	12.65 ± 1.33[b]	3.79 ± 0.62[a]
Zinc (Zn)	108.00 ± 1.00[a,b]	27.00 ± 7.09[a]	123.67 ± 1.33[b]
Strontium (Sr)	3.00 ± 0.12[b]	14.30 ± 0.56[c]	1.32 ± 0.26[a]
Iron (Fe)[d]	30.67	19.00	26.67
Manganese (Mn)[d]	1	1.33	<1
Cadmium (Cd)[d]	0.01	<0.01	0.03
Nickel (Ni)[d]	0.43	1.69	0.73
Lead (Pb)[d]	0.03	0.11	<0.01
Silver (Ag)[d]	<0.01	<0.01	<0.01
Mercury (Hg)	ND	ND	ND
Arsenic (As)	ND	ND	ND

Mean values and SE presented in the table.
[a,b,c]Different letters within a row indicate statistical difference at $P < 0.05$; IPR, immature pollock roe; SP, soluble protein fraction of IPR; IP, insoluble protein fraction of IPR.
[d]Average values listed; for calculation purposes, detectable limit values were used for values below the detectable limit.
No statistical analysis done on minerals where some values were below detectable limits.
ND, not detected.

Physical and functional properties

Fresh immature pollock roe was a light colored material when first collected and retained its light color when dried (L^* 81.5; a^* 3.2; b^* 19.6) as shown in Table 5. The protein powder fractions made from pollock roe had higher L^* values of 87.5 (SP) and 88.4 (IP) and lower a^* values than IPR. However, L^* and a^* values were not significantly different among all the 3 samples. All protein powders were light yellow in color; however, the source of the yellow color was not determined. The b^* value for SP was higher than IP ($P < 0.05$), indicating more yellowness, but was not significantly higher than IPR.

Protein solubility values for IPR, SP, and IP were all significantly different ($P < 0.05$; Table 5). The solubility value of 10.1% for IPR was lower than values reported for pollock and cod heads, frames and fillets where values ranged from 10.2% to 16.3% (Bechtel 2003) and much lower than viscera. The solubility of the SP fraction was 59.0%, which was much higher than the IP fraction (1.6%). The amino acid composition indicated that the SP sample had a higher proline content and also lower levels of essential amino acids, consistent with a protein fraction containing structural protein components such as collagen fragments. The large difference in percent solubility between SP and IP was expected because these 2 fractions were separated from one another by centrifugation after IPR was heated to 85 °C.

Aside from nutritional attributes, functional properties influence the usefulness of an ingredient. An important attribute of a protein ingredient is the ability to form a stable emulsion. The emulsifying stability (ES) of all samples ranged from 63.6% to 71.3%. The SP sample had the highest ES value ($P < 0.05$), which could be duly attributed to the greater solubility of the protein fraction. As shown in the gel in Figure 1 the SP sample did not have the same banding profile as the IPR and IP fractions. The SP samples appeared to have fewer discrete protein bands and also lower molecular weight peptide below 20 KDa in size. ES values of 64.5% to 66.4 were reported for protein powders made from herring by-products (Sathivel and others 2004). However, when protein powders were made from hydrolyzed herring by-products, lower ES values (48.6% to 54.2%) were reported (Sathivel and others 2003).

Fat binding/adsorption capacity is one of the important functional characteristics of ingredients used in the meat and confectionery industries. SP exhibited a fat absorption value approximately double ($P < 0.05$) that of IPR and IP. The values of 2.8 and 3.5 mL of oil/g protein for IP and IPR were in the lower range as compared to the values reported in the following 2 studies—Sathivel and others (2003, 2004) reported that fat adsorption capacity values ranged from 3.9 to 11.5 mL of oil/g protein for herring protein powders and 3.7 to 7.3 mL of oil/g protein for hydrolyzed herring byproduct proteins. Kristinsson and Rasco (2000) reported values of 2.86 to 7.07 mL of oil/g protein for Atlantic salmon protein hydrolysates.

SDS electrophoresis of IPR indicated a major protein band with molecular weight of 103 KDa and a number of other discreet protein bands with molecular weights estimated at 90 and 13 KDa in all samples (Figure 1). IP had a large amount of the 103 KDa protein and smaller amounts of proteins that could also be identified in the IPR sample. In addition, bands with molecular weight greater than 103 KDa are present in IP but not IPR and could possibly be aggregated protein formed during the fractionation process. The process of making the SP fraction was to heat the IPR and then separate the SP and IR fractions by centrifugation. The SP fraction contained large amounts of stained material in the 13 KDa and lower molecular weight range area and above and below the 103 KDa band.

Table 5—Physical and functional properties of immature pollock roe (IPR) and the soluble and insoluble protein fractions of IPR

	IPR	SP	IP
Color			
L^*	81.47 ± 0.14[a]	87.49 ± 1.78[a]	88.43 ± 0.17[a]
a^*	3.20 ± 0.06[a]	0.57 ± 1.35[a]	1.01 ± 0.09[a]
b^*	19.63 ± 0.37[a,b]	26.93 ± 1.74[b]	12.79 ± 0.27[a]
Percent solubility	10.13 ± 0.73[b]	58.98 ± 3.53[c]	1.57 ± 0.07[a]
Emulsifying stability[d]	65.33 ± 0.96[a]	71.33 ± 3.67[a]	63.60 ± 1.20[a]
Fat adsorption capacity[e]	3.50 ± 0.08[b]	6.42 ± 0.23[c]	2.78 ± 0.26[a]

Mean values and SE presented in the table.
[a,b,c]Different letters within a row indicate statistical difference at $P < 0.05$; IPR, immature pollock roe; SP, soluble protein fraction of IPR; IP, insoluble protein fraction of IPR.
[d]Percent emulsified.
[e]Milliliters of oil/g of protein.

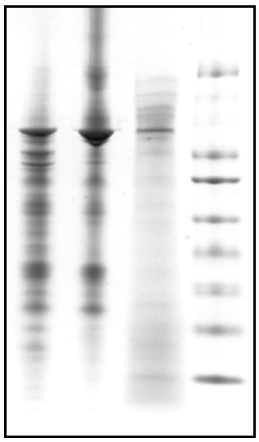

Figure 1—Sodium dodecyl sulfate (SDS)—tricine/polyacrylamide gel electrophoresis profiles of freeze-dried immature walleye pollock roe (IPR; lane 1), insoluble protein (IP; lane 2), soluble protein (SP; lane 3), and standard with molecular weight of 8 to 210 KDa (lane 4)

Conclusions

Immature pollock roe had a high protein and low fat content. The lipid fraction contained high levels of DHA and EPA and about 71% lipid was phospholipids. Protein from dried IPR was of high quality and had a TEAA/TNEAA ratio of 0.77. The protein was heated and fractionated into soluble and insoluble products. The TEAA/TNEAA ratio of the IP product was similar to that of IPR and better than the soluble product. The soluble product in addition to having a high degree of solubility had better fat adsorption capacity than either IPR or the IP. These results indicated that protein ingredients, which have unique nutritional and functional properties, can be produced from immature pollock roe.

Acknowledgments

We thank the U.S. Dept. of Agriculture (Agriculture and Research Services) for providing the funds for this research project, and the Fishery Industrial Technology Center for providing equipment funding. We also thank Heather Averett, Steven Coen, Kathryn Brenner, and Mary Patterson for their technical expertise. This research is part of an ongoing effort to enhance utilization of Alaska fish by-products by developing novel food and feed ingredients.

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